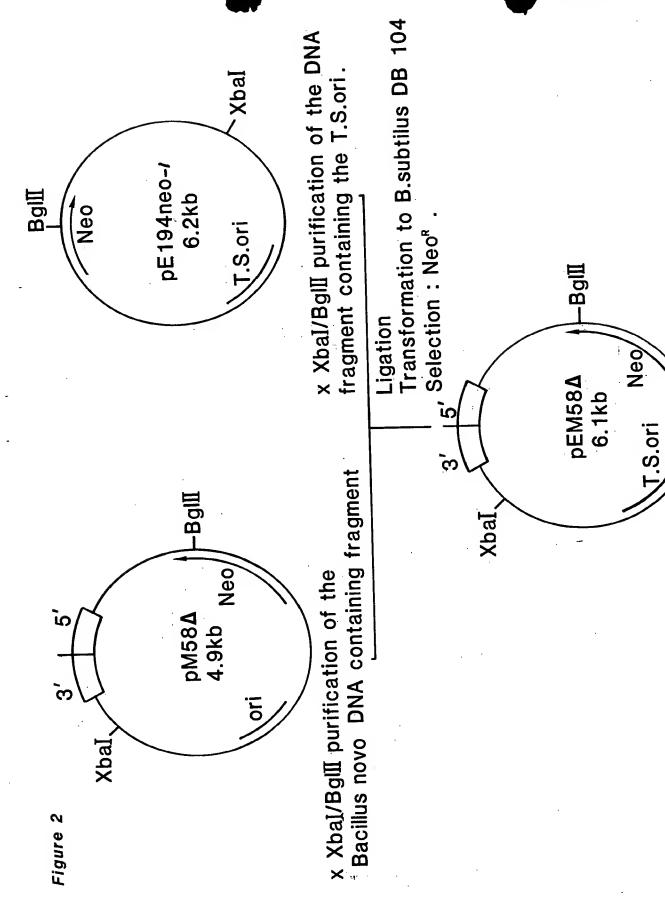
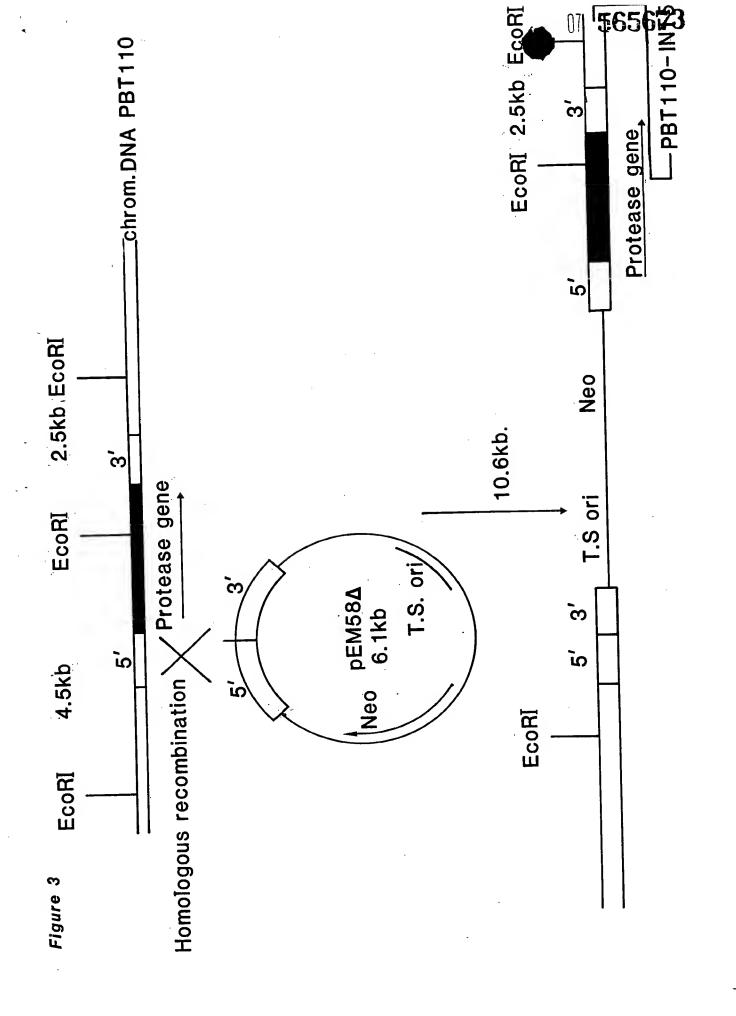
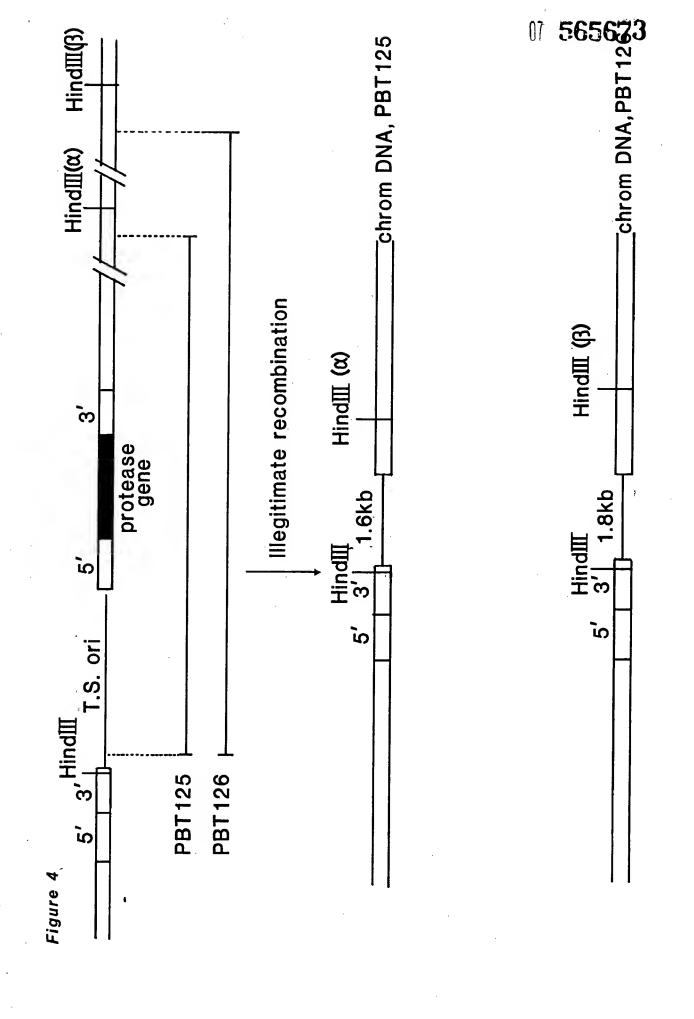


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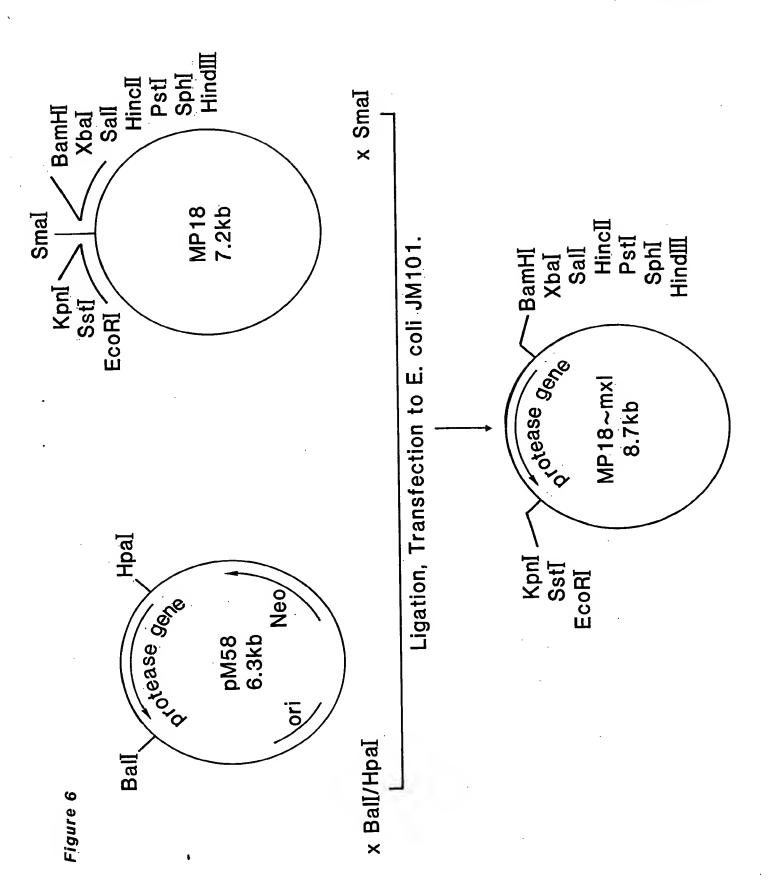


Hindiii . 40 AAGCTTTTCAAATGATCCAGGAC AAAACGATTTTTTGAGGAAAGTTATAAAT TTCCGAACGATATGGCAAGC
. 120
. XbaI . 360 400 AAAAAGAGATTGATTT <u>TCTAGA</u> AAATGAATACGGTAAGGAAAATATGCTGTATGCGACTGTCCATCTGGATGAAAGAGTC
600 640 TTACAGAACGAGAACATAAAGCGATGGATCAGTACAAGAAGATACTGTATTTCATAAACAGGAACTGCAAGAAGTTAAG
800 TGAGCGTACAGGTTTGTTCTCTGGACGTGAAGAGACTGGTAGAAAGATATTAACTGCTGATGAATTTGAACGCCTGCAAG
. PstI . 840 880 AAACAATCTCTTCTGCAGAACGATTGTTGATGATTACGAAAATATTAAGAGCACAGACTATTACACAGAAAATCAAGAA
920 960 TTAAAAAAACGTAGAGAGAGTTTGAAAGAAGTAGAGTGAATACATGGAAAGAGGGTTACCGAAAAAAAGTAAAGAGGTTAA
1000 1040 TAAATTAAAGCGAGAGAATGTTGAATGAGCAGTTGAATGTATCAGAGAAATTTCAAGCTAGTACAGTGACTTTAT
1080 1120 ATCGTGCTGCGAGGGCGAATTTCCCTGGGTTTGAGAAAGGGTTTAATAGGCTTAAAGAGAAATTCTTTAATGATTCCAAA
. 1240
. 1320
. 1400
GGTTATTTAGGGGAGAAAACATAGGGGGGTACTACGACCAAAATGGAGGAATACGGCAGCGCGAGCATGGTGGATGCTTG
. 1560
HindIII

HindIII TGAGGTG<u>AAGCTT</u>

figure 5A

HindIII AAGCTTTTCAAATGATCCAGGAGA AAACGAT	40 FTTTTTGA	GGAAAGTTATAAATT	TCCGAACGATATGGCAAG
AAAATATTGCTTATGCAACAGTTCATAATGATGA	120 GCAAACCC	CTCACATGCATTTAGGT	. 160 STTGTGCCTATGCGTGATGG
AAACTGCAAGGAAAAAATGTGTTTAATCGTCAAGA	200 AACTGTTA	TGGCTACAAGATAAATT	. 240 CCCGAGCATATGAAAAAACA
GGGTTTTGAGTTGAAGCGTGGTGAACGTGGCTCTC	280 GACCGTAA	ACATATTGAGACAGCTAA	320 ATTTAAAAAACAAACTTTG
. XbaI . AAAAAGAGATTGATTT <u>TCTAGA</u> AAATGAATACGG	360 raaggaaa	ATATGCTGTATGCGACTC	400 TCCATCTGGATGAAAGAGT
. CCACATATGCACTTTGGTTTTGTCCCTTTAACAGA	440 AGGACGGG	AGATTGTCTGCAAAAGAA	. 480 CAGTTAGGCAACAAGAAAG
CTTTACTCAATTACAAGATAGATTTAATGAGTAT	520 GTGAATGA	GAAAGGTTATGAACTTGA	. 560 AAGAGGCACGTCCAAAGAG
TTACAGAACGAGAACATAAAGCGATGGATCAGTAG	600 CAAGAAAG	ATACTGTATTTCATAAAC	. 640 AGGAACTGCAAGAAGTTAAC
GATGAGTTACAGAAGGCAAATAAGCAGTTACAGAG	680 gtggaata	GAGCATATGAGGTCTACC	720 GAAACCCTTTGATTATGAAAA
TGAGCGTACAGGTTTGTTCTCTGGACGTGAAGAGA	760 ACTGGTAG	AAAGATATTAACTGCTGA	. 800 TGAATTTGAACGCCTGCAA
. PstI . AAACAATCTCTT <u>CTGCAG</u> AACGGATTGTTGATGAT	840 PTACGAAA	ATATTAAGAGCACAGACI	880 ATTACACAGAAAATCAAGAA
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TAAATTAAAGCGAGAGAATGATAGTTTGAATGAG	1000 CAGTTGAA	TGTATCAGAGAAATTTCA	. 1040 AGCTAGTACAGTGACTITA
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TAAGCGAAAGTAGTAGCGACAGCTATTAACTTTCC	1320 GGTTGCAA	AGCTCTAGGATTTTTAAT	. 1360 GGACGCAGCGCATCACACGC
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AGCAGGCA.100BPTTCAGTCCGTC	1560 CAAGAGAT	GCGCAACTACGATAACTC	. 1600 CATTCTTTATGGTATGGCTC
AGAAAATAGCGATTATGAACGTCGTTGGCGGTACA	1640 ATTTACCG	ACGAGCCATATGGGGATI	GCTGTCCGCAAACGGCGACC
ECORI	1720 CGAGACTT		figure 5B



30 60 AATTCACCTC GAAAGCAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG ATTTCAACG TGAAAAATT ATTATTCGCA ATTCCAAGCT AATTCACCTC 150 180 GAAAGCAAGC TGATAAACCG ATACAATTAA AGGCTCCTTT TGGAGCCTTT TTTTTTGGAG 210 240 ATTTTCAACG TGAAAAAATT ATTATTCGCA ATTCCAAGCT CTGCCTCGCG CGTTTCGGTG 300 ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG 360 CGGATGCAGA TCACGCGCCC TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC 390 GCAGCGTGAC CGCTACACTT GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT 450 480 CCTTTCTCGC CACGTTCGCC GGCTTTCCCC GTCAAGCTCT AAATCGGGGG CTCCCTTTAG 540 GGTTCCGATT TAGTGCTTTA CGGCACCTCG ACCCCAAAAA ACTTGATTAG GGTGATGGTT 570 CACGTAGTGG GCCATCGCCC TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT 630 660 TCTTTAATAG TGGACTCTTG TTCCAAACTG GAACAACACT CAACCCTATC TCGGTCTATT 690 CTTTTGATTT ATAAGGGATT TTGCCGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT 750 780 AACAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT TACAATTTGA TCTGCGCTCG 810 840 GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA 870 GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAA GCCAGCAAAA GGCCAGGAAC 930 960 CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCTGA CGAGCATCAC 990 1020 AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG 1050 1080 TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC

CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC AATGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TGCAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC

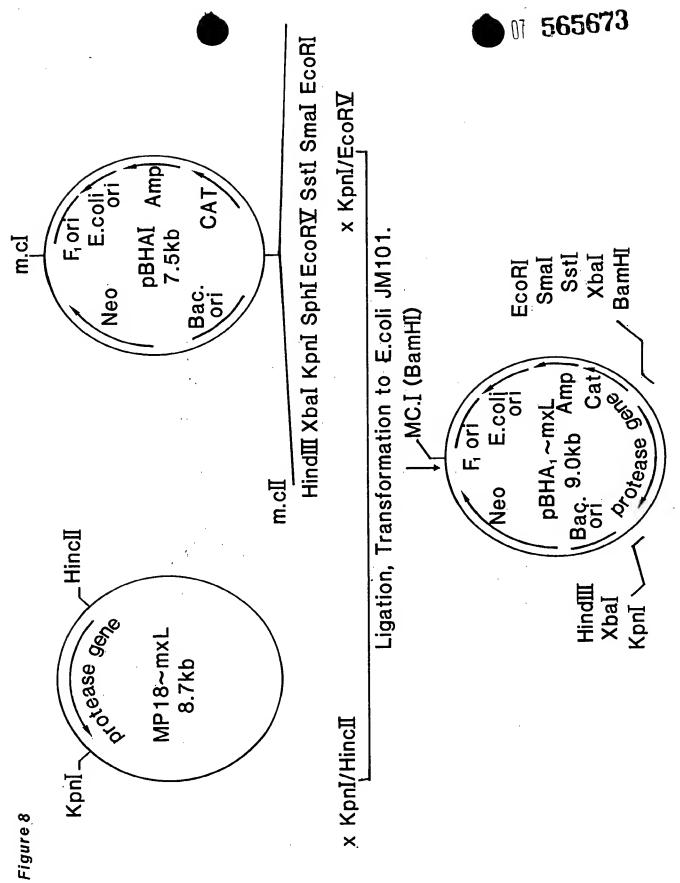
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ACCAGCGTTT	CTGGGTGAGC	2490 AAAAACAGGA	AGGCAAAATG	CCGCAAAAAA	2520 GGGAATAAGO
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CATTAGGCGG	GCTCGAATTT	2790 CTGCCATTCA	TCCGCTTATT	ATCACTTATT	2820 CAGGCGTAGO
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CATGATGAAC	CTGAATCGCC	2970 AGCGGCATCA	GCACCTTGTC	GCCTTGCGTA	3000 TAATATTTGO
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TGAAACTCAC	CCAGGGATTG	3090 GCTGAGACGA	AAAACATATT	CTCAATAAAC	3120 CCTTTAGGGA
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CGGTGTAACA	AGGGTGAACA	3270 CTATCCCATA	TCACCAGCTC	ACCGTCTTTC	3300 ATTGCCATAC
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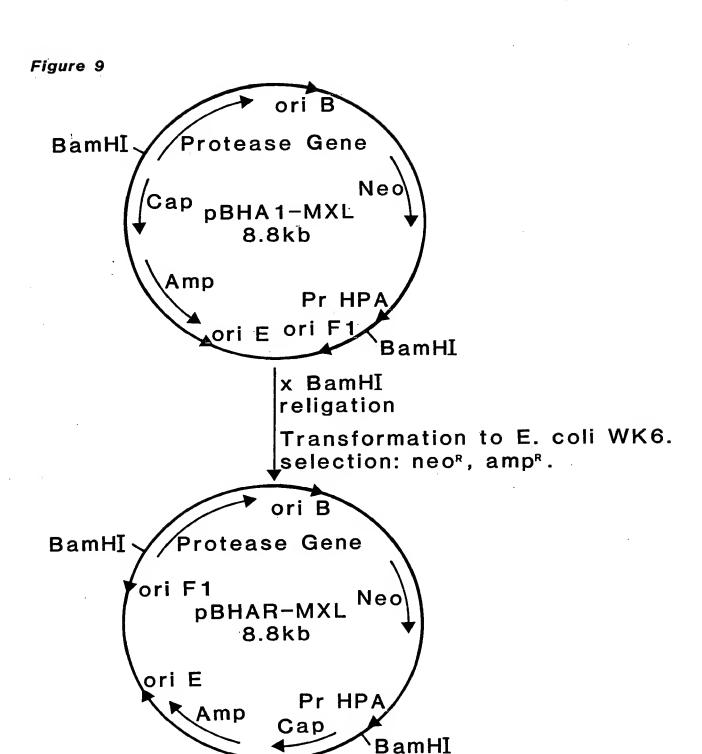
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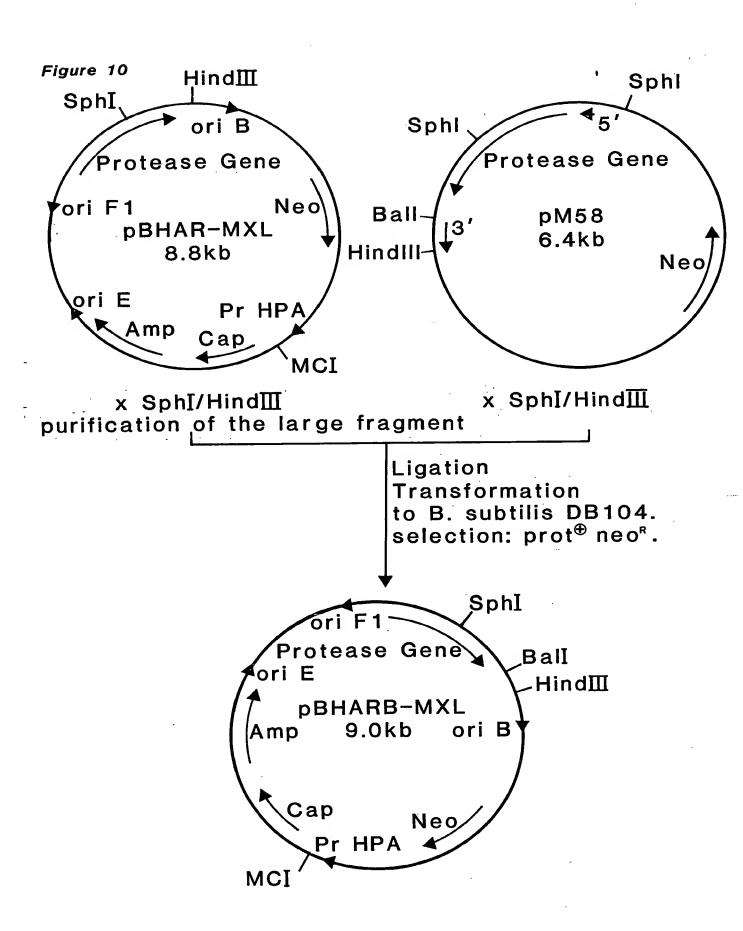
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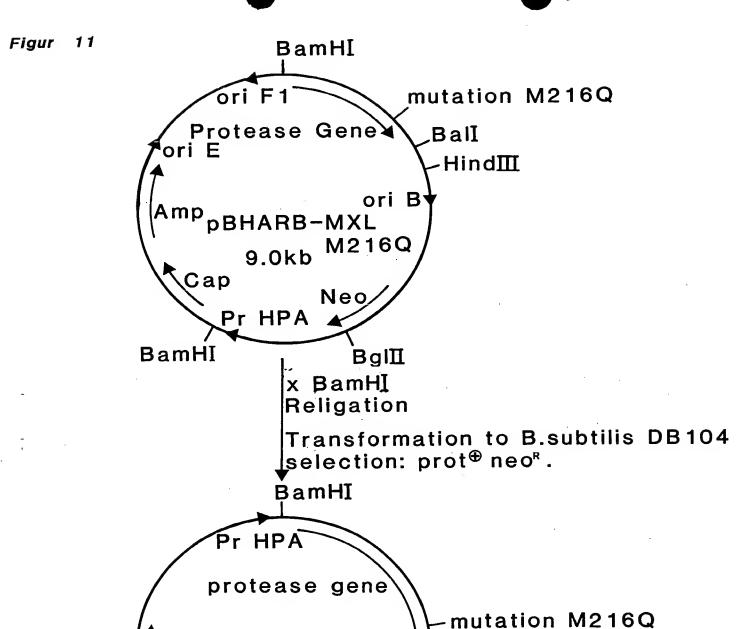
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6810 6840 TCTTGTATCT TTTTTATTTT GAGTGGTTTT GTCCGTTACA CTAGAAAACC GAAAGACAAT 6870 AAAAATTITA TTCTTGCTGA GTCTGGCTTT CGGTAAGCTA GACAAAACGG ACAAAATAAA 6930 6960 AATTGGCAAG GGTTTAAAGG TGGAGATTTT TTGAGTGATC TTCTCAAAAA ATACTACCTG 6990 7020 TCCCTTGCTG ATTTTTAAAC GAGCACGAGA GCAAAACCCC CCTTTGCTGA GGTGGCAGAG 7050 7080 GGCAGGTTTT TTTGTTTCTC TTTTCTCGTA AAAAAAAGAA AGGTCTTAAA GGTTTTATGG 7110 7140 TTTTGGTCGG CACTGCCGAC AGCCTCGCAG GACACACAT TTATGAATAT AAAGTATAGT 7170 GTGTTATACT TTACTTGGAA GTGGTTGCCG GAAAGAGCGA AAATGCCTCA CATTTGTGCC 7230 7260 ACCTAAAAAG GAGCGATTTA CATATGAGTT ATGCAGTTTG TAGAATGCAA AAAGTGAAAT 7290 7320 CAGGGGGATC CTCTAGAGTC GAGCTCAAGC TAGCTTGGTA CGTACCAGAT CTGAGATCAC GCGTTCTAGA GGTCGA









Ball

HindⅢ

pBHB-MXL M216Q

5.2kb

ori B

BgI∏

Neo

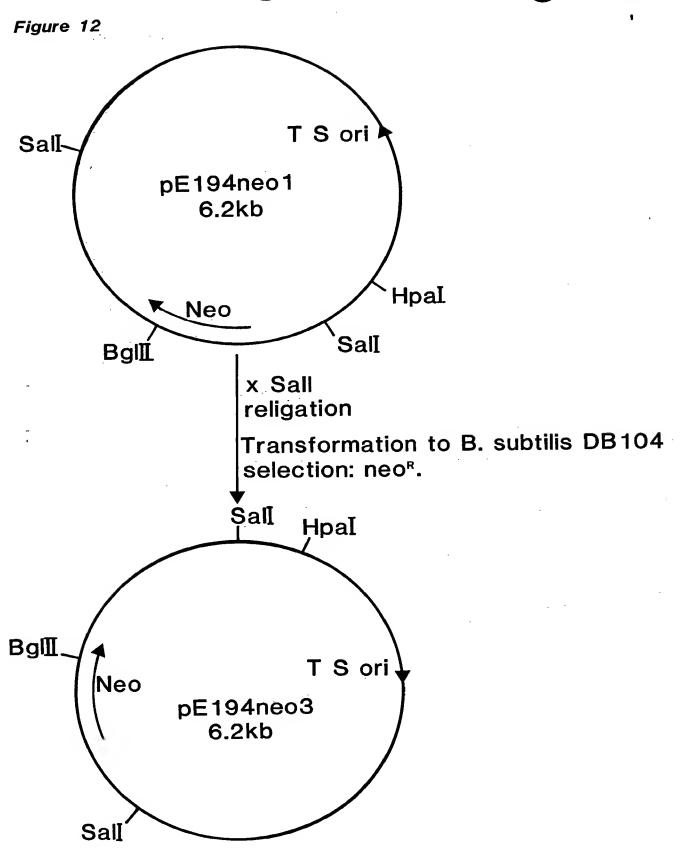
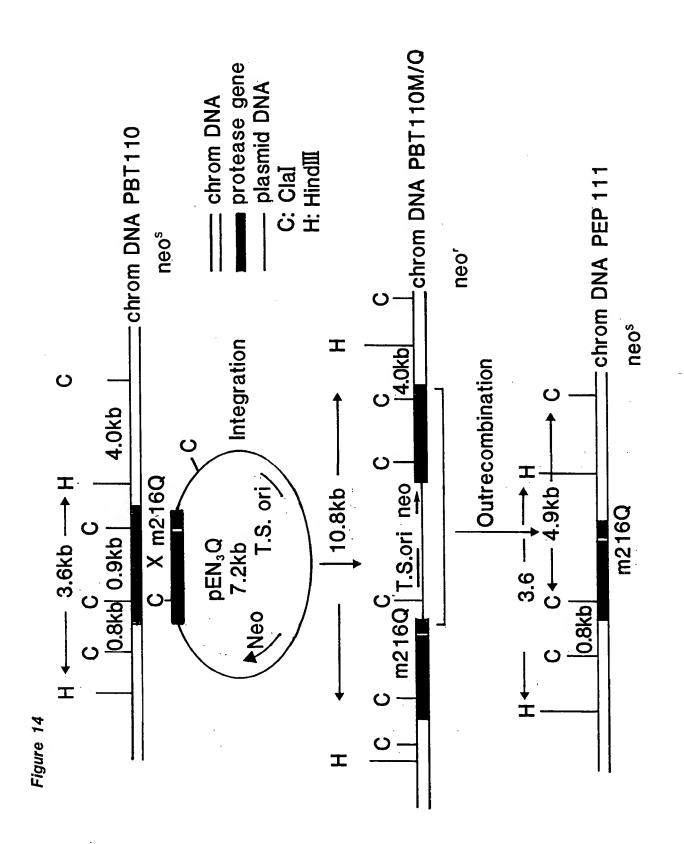


Figure 13 **ḤpaI** Pr HPA mutation Protease Gene m216Q Bgl T.S.ori pBHB-MXL M216Q Bg∭pE194neo3 Neo 5.2 kb 6.2kb Neo Ball ori B x Ball/BglII x Hpal/Bgll Ligation Transformation to B.subtilis DB104. selection: prot[®] neo^R. Pr HpaII mutation M216Q Protease Gene Bgl∏. -Ball/Hpal Neo pEN3Q 7.2kb T.S.ori





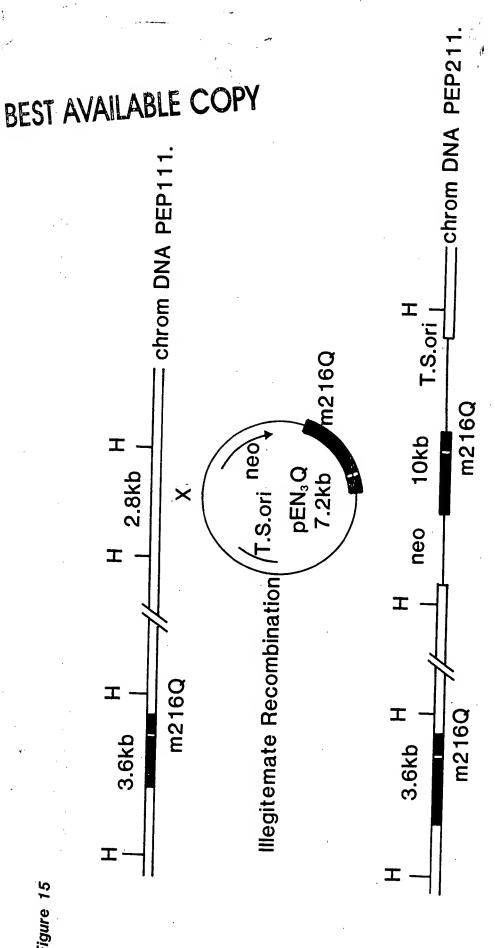


Figure 15